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AtJ6, a unique J-domain protein from *Arabidopsis thaliana*☆

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Abstract

An *Arabidopsis thaliana* cDNA encoding a protein, AtJ6, related to *Escherichia coli* DnaJ was sequenced. Translation of the AtJ6 nucleotide sequence yields a protein with an N-terminal J-domain, but which lacks the G/F, and C-rich domains characteristic of DnaJ. In addition to the J-domain, one region of the sequence has homology with the prokaryotic FtsA protein. The remainder of the AtJ6 sequence is not notably related to anything in the sequence databases. The AtJ6 sequence represents a unique member of the *A. thaliana* J-domain protein family. The results of Northern analysis revealed that the AtJ6 mRNA is expressed in all organs of *A. thaliana*; expression was high in leaves, flowers, and siliques, and low in roots. The results of Southern analysis are consistent with a single unique gene.

Keywords: cDNA; Chaperone; Exon; Expression; Gene; Intron; Nucleotide sequence

1. Introduction

The 70 kD stress proteins comprise a ubiquitous set of highly conserved molecular chaperones [1,2]. The Stress70 proteins do not, however, function as chaperones by themselves but rather in concert with two accessory or co-chaperone proteins. The functional association of these components is often referred to as the Stress70 chaperone 'machine' [2,3]. The archetypical Stress70 chaperone machine was defined in *Escherichia coli*, and consists of the products of *dnaK* (Stress70), *dnaJ* and *grpE*. DnaK is the central, ATP-dependent component of the machine, and functions as a chaperone in association with DnaJ, an activating protein, and GrpE, a nucleotide exchange factor [4,5].

DnaJ was first isolated as a 41 kDa heat shock protein from *E. coli* [6]. It was subsequently reported that DnaJ was involved with DNA replication, stimulating the capacity of DnaK to form a replication-competent complex at the phage origin of replication. It has been demonstrated that DnaJ stimulates the rate of hydrolysis of DnaK-bound ATP [4]. This stimulation by DnaJ is similar in concept to the effect of the GTPase activating proteins upon the low molecular weight GTP-binding proteins [7]. In addition to co-operating with DnaK, it has been demonstrated that DnaJ can function independently as a chaperone [3].

It was subsequently shown that homologues of DnaJ are present in all of the compartments of eukaryotic cells [8]. The eukaryotic DnaJ homologues also function as co-chaperones in conjunction with DnaK homologues (70 kD stress proteins). Furthermore, roles for DnaJ homologues in signal transduction [9] and controlled proteolysis [10] have been described. Flowering plant cells are particularly rich in DnaJ homo-

Abbreviations: Stress70, any of the 70 kDa family of stress related proteins.

☆ The GenBank accession number for the sequences reported in this article is AF037168.

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logues, perhaps in part due to the extensive compartmentation. We are in the process of cloning and characterizing cDNAs for all of the DnaJ homologues from the model plant *Arabidopsis thaliana* [11–13]. Herein we report the sequence of a cDNA encoding a unique J-domain protein, AtJ6.

2. Materials and methods

2.1. Reagents

Unless otherwise noted, DNA modifying enzymes were from New England BioLabs (Beverly, MA) and were used according to the manufacturers recommendations. All buffers were from Research Organics, Inc. (Cleveland, OH). Other biochemicals were from the Sigma Chemical Company (St. Louis, MO) and were of the highest purity available.

2.2. Biological materials

An Arasystems kit, and seeds of *A. thaliana* WT-2 var. Columbia were from Lehle Seeds (Round Rock, TX). Seeds were germinated according to the supplier's suggestions. Seedlings were grown in a Percival (Boone, IA) growth chamber at 23°C and a 14 h photoperiod of 200 $\mu E/m^2$ per s photosynthetically active radiation.

2.3. Nucleic acids

An EST clone, ID 161j18t7, was obtained from the Arabidopsis Biological Resource Center, The Ohio State University. This clone is a random *Sal*I × *Not*I fragment inserted into pZL1. An *A. thaliana* cDNA library (leaves of 4.5 week, light-grown plants, var. Columbia) in λgt11 was from Clontech (Palo Alto, CA). Oligonucleotides were prepared using an Applied Biosystems Model 381a

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ggaacacggaaatattggcgcccagataatcttcttctgtgaagcattttaagtcgccga 60
ctccaatcaacagagcccagttattccattttatcgaattaattagggttcagagagATG 120
                                                          M
GGTAGGAAAAAGAAATCTAGGGCTTCGACAACCGAAGAAGATGAGATTGAGATGGATAAT 180
 G  R  K  K  K  S  R  A  S  T  T  E  E  D  E  I  E  M  D  N
GCTGGCCCATCCTCTGAGACAAGTCTTTACGAGGTTCTTGGAGTTGAAAGAAGAGCCACT 240
 A  G  P  S  S  E  T  S  L  Y  E  V  L  G  V  E  R  R  A  T
TCACAGGAAATAAGAAAAGCGTACCATAAGTTGGCATTGAAGCTTCACCCTGATAAAAAT 300
 S  Q  E  I  R  K  A  Y  H  K  L  A  L  K  L  H  P  D  K  N
CAGGATGATAAGGAAGCTAAAGACAAGTTCCAGCAGCTGCAAAAAGTTATATCAATTCTT 360
 Q  D  D  K  E  A  K  D  K  F  Q  Q  L  Q  K  V  I  S  I  L
GGTGATGAAGAGAAAAGGGCAGTCTATGATCAAACTGGCTCAATTGATGATGCTGATATT 420
 G  D  E  E  K  R  A  V  Y  D  Q  T  G  S  I  D  D  A  D  I
CCTGGAGATGCGTTTGAGAATTTGCGGGATTTCTTCCGGGACATGTATAAGAAGGTCAAC 480
 P  G  D  A  F  E  N  L  R  D  F  F  R  D  M  Y  K  K  V  N
GAAGCTGATATTGAAGAGTTTGAGGCAACCTACAGGGGATCTGAGTCAGAGAAGAAAGAC 540
 E  A  D  I  E  E  F  E  A  T  Y  R  G  S  E  S  E  K  K  D
TTGCTTGAGCTTTTCAACAAGTTTAAGGGTAAAATGAACAGGCTATTCTGCTCAATGCTT 600
 L  L  E  L  F  N  K  F  K  G  K  M  N  R  L  F  C  S  M  L
TGCTCGGACCCCAAGCTTGATTCACACCGTTTCAAAGACATGCTTGATGAGGCCATTGCA 660
 C  S  D  P  K  L  D  S  H  R  F  K  D  M  L  D  E  A  I  A
GCAGGAGAAGTGAAGTCAAGCAAGGCATATGAGAAATGGGCAAATAAAATTTCAGAAACG 720
 A  G  E  V  K  S  S  K  A  Y  E  K  W  A  N  K  I  S  E  T
AAACCGCCCACCAGTCCATTGAGGAAGAGGAAGAAGAAGAAGTCAGCACCCAAGGATTCA 780
 K  P  P  T  S  P  L  R  K  R  K  K  K  K  S  A  P  K  D  S
GAGACAGATCTTTGCTTGATGATTGCGAAACGACAAGAGGAGAGGAAAGGGAAGGTGGAC 840
 E  T  D  L  C  L  M  I  A  K  R  Q  E  E  R  K  G  K  V  D
TCGATGTTTTCATCACTTATCTCTAGGTATGGTGGTGATGCGGAAGCAGAGCCCACTGAA 900
 S  M  F  S  S  L  I  S  R  Y  G  G  D  A  E  A  E  P  T  E
GAAGAATTTGAAGCTTCCCAGAGAAGGATTGAAACCCAAAGAAAACCATCCAAGAAGTCT 960
 E  E  F  E  A  S  Q  R  R  I  E  T  Q  R  K  P  S  K  K  S
AGAGGAAAGTAGaggtcttaaagcagagttgtcttctagtagtatttgtgatggaacaag 1020
 R  G  K  *
aatgtttatttctttccaaatttcatcaatttccaaaatcagtaaatatttcgattatt 1080
gctttataaattgaggaattatcttctccaaaaaaaaaaaaaaaaaaaaaa         1132
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Fig. 1. The AtJ6 nucleotide and deduced amino acid sequences. Non-coding regions are lower case italic letters while the reading frame is roman capital letters. The J-domain is underlined, and the HPD β-turn motif is presented in bold typeface. The potential nuclear localization sequences are bold italic. These sequences appear in GenBank under the accession number AF037168.

A

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1    TSLYEVLGVERRATSQEIRKAYHKLALKLHPDKNQDDKEAKDKFQQLQKVISILGDEEKRAVYDQTG94
2    RNYYDVLGVSPKATREEIKKSFHELAKKFHPDTNRNNPSAKRKFQEIREAYETLGNSERREEYDKLQ113
3    TKFYEILGVPKTAAPEDLKKAYKKAAIKNHPDKGGDPE....KFKELAQAYEVLSDPEKREIYDQYG75
4    RDYYEVLGVSKSASKDEIKKAYRKLSKKYHPDINKEAGSDE.KFKEVKEAYETLSDDQKRAHYDQFG69
5    QDYYEILGVSKTAEEREIRKAYKRLAMKYHPDRNQGDKEAEAKFKEIKEAYEVLTDSQKRAAYDQYG70
6    YYEVLGVQRHASPEDIK..KAYRKLALKWHPDKNPENKEEAERKFKQVAEAYEVLSDAKKRDIYDKY68
7    KDPYDTLGLKKSATGAEIKKAYYKLAKKYHPDINKEPDAEK.KFHDLQNAYEILSDETKRQQYDQFG125
8    QDYYAILEIDKDATEKEIKSAYRQLSKKYHPDKNAGSEEAHQKFIEVGEAYDVLSDPEKKKIYDQFG115
Consensus DYYE+LGV K+AT  EIKKAY+KLAKKYHPDKN +  EA  KF+E+ EAYE+LSD EKR  YDQ+G
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B

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1    YTELL-NLVNEEILQLQEKLRQQGVKHHLAAGIVLTGGAAQIEGLAACAQRVFHTQVRIG359
2    LAEVISARAEEILSMVKAEYDKATKGQFEVFRVVITGGGSQLRGLKELSNKIFAKQSRIG353
3    VSRIVRARIEETLELIRDRIQRSGFSPIVGKRIVLTGGASQLTGLPEAARRILARNVRIG380
4    LSEIISA-RIKHILEQIKQELDRRRLLDLPGGIVLIGGNAILPGMVELAQEVFGVRVKLY346
5    ADIPGDA-FENLRDFFRDMYKKVTEADIEEFEATYRGSESEKKDLLELFNKFKGKMNRLF157
Consensus ++E++ A  EE +  +++          +   IVLTGG+SQL GL ELA+++F+  VRIG
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Fig. 2. AtJ6 sequence comparisons. (A) Alignment of the AtJ6 J-domain with those of *E. coli* DnaJ and selected homologues. The sequences compared are: (1) AtJ6 (GenBank accession AF037168); (2) *A. thaliana* AtJ1 (U16246); (3) *A. thaliana* AtJ2 (L36113); (4) *E. coli* DnaJ (M12565); (5) *Homo sapiens* HDJ2 (AF080569); (6) *Saccharomyces cerevisiae* MDJ1p (Z28336); (7) *S. cerevisiae* SCJ1p (X58679); (8) *Bacillus subtilis* DnaJ (M84964). If at least four of the eight sequences are identical, this is the consensus. If at least four residues are similar, this is indicated by (+). The HPD β-turn motif is presented in bold typeface. (B) Alignment of the regions of sequence homology between AtJ6 and bacterial FtsA proteins. Sequences compared are: (1) *E. coli* (K02668); *Rickettsia prowazekii* (AJ235271); (3) *Sinorhizobium meliloti* (AF024660); (4) *Streptococcus pneumoniae* (AF068910); (5) AtJ6. If at least three of the sequences are identical, this is the consensus. If at least three residues are similar, this is indicated by (+). Spaces (.) have been inserted to optimize homology. Clustal alignments were performed using the DNAMAN sequence analysis program, version 2.71.

synthesizer using the phosphoramidite, trityl-off program, then purified by precipitation.

2.4. DNA sequencing

Sequencing was performed using an Applied Biosystems *Taq* DyeDeoxy Terminator Cycle Sequencing kit and a Model 370A Applied Biosystems Automated DNA Sequencer. The sequences of internal primers were based upon 3'-proximal regions of previously determined sequences. These 20-mer sequencing primers were prepared using the Applied Biosystems Synthesizer. DNA sequence analysis and manipulation, and comparison with published sequences, were accomplished using the program DNAMAN, version 2.71, from Lynnon BioSoft, Vaudreui, Quebec, Canada.

2.5. Northern and southern analyses

Total RNA was isolated from various organs of *A. thaliana* using the materials and protocol supplied by Clontech Laboratories (Palo Alto, CA) with the Extract-A-Plant RNA isolation kit. Poly A^+RNA was separated from total RNA using the mRNA Separator kit from Clontech. Electrophoresis of RNA through formaldehyde-containing gels, transfer to Amersham Hybond N nylon membranes, and hybridization with DNA probes were as previously described [11]. Genomic DNA was isolated from *A. thaliana* leaves, restriction digested, separated by agarose gel electrophoresis, and transferred to nylon membranes as previously described [11]. The complete AtJ6 cDNA was used as a probe, and was labeled with [^{32}P]dCTP using the Random Primer DNA Labeling Kit from Bio-Rad Laboratories (Richmond, CA). This same probe was used to detect RNA on Northern blots and DNA on Southern blots. Images from gels/films/membranes were captured using a MICROTEK MRS-1200E6 flat bed scanner, and imported directly into the Microsoft PowerPoint graphics program.

3. Results and discussion

3.1. Characteristics of the nucleotide and deduced amino acid sequences

The 1132 bp cDNA was sequenced completely in both directions (Fig. 1). The clone comprises 117 bp of 5′-UTR, an 852 bp reading frame, and 163 bp of 3′-UTR. The 3′-UTR ends in a 23 base poly-A tail. The cDNA reading frame encodes a polypeptide of 284 amino acids with a molecular weight of 32 603 Da and a pI of 8.4 (Fig. 1).

3.2. Homology with DnaJ

Amino acids 19 through 91 of AtJ6 define the J-domain, a region thought to have a direct physical interaction with the Stress70 component of the chaperone machine [2,14,15]. The HPD β-turn motif, that is both diagnostic of and absolutely essential for DnaJ function, is present in the same relative position within the AtJ6 sequence as is found in DnaJ and most eukaryotic homologues (Fig. 1). The AtJ6 J-domain sequence was aligned with those of selected DnaJ homologues (Fig. 2A),

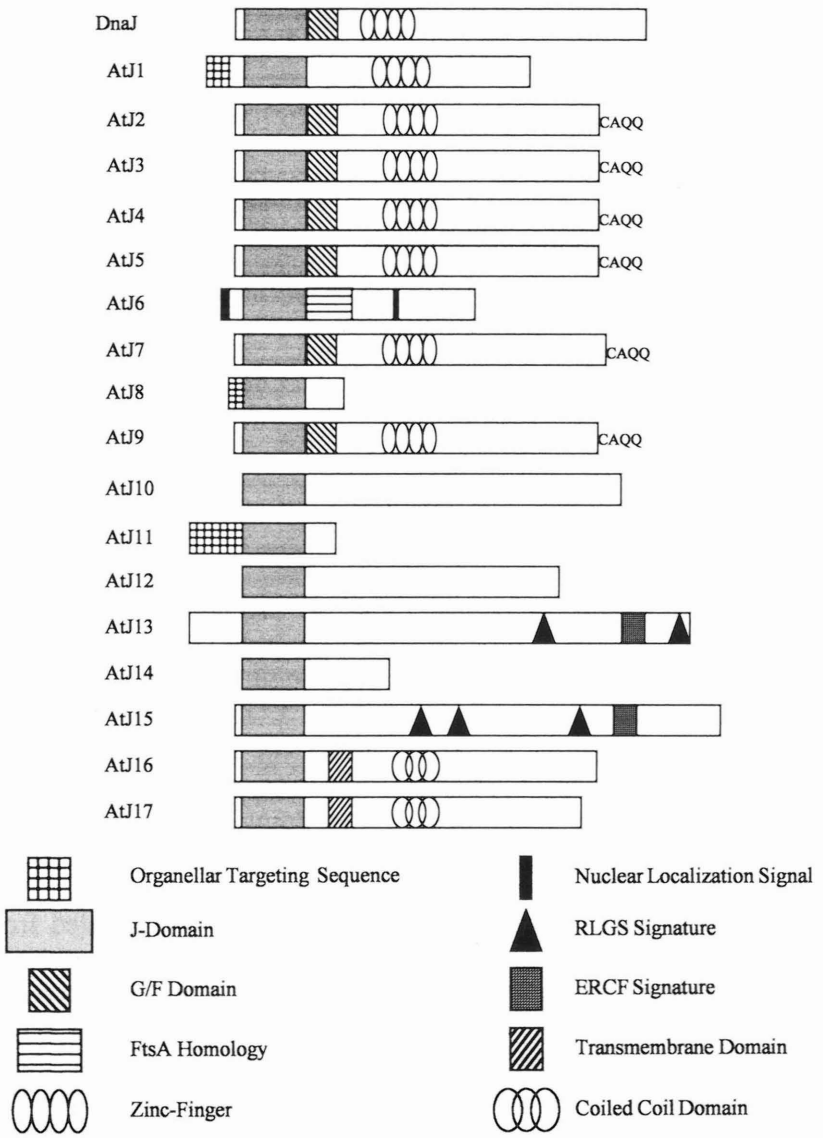

Fig. 3. Schematic presentation of the *A. thaliana* J-domain proteins, indicating the modular organization. *E. coli* DnaJ is included as the archetype, showing the relative positions of the J-, G/F- and zinc finger-domains. *E. coli* DnaJ, (GenBank accession K02668); AtJ1, (U16246) [11]; AtJ2, (L36113) [12]; AtJ3, (U22340) [13]; AtJ6, (AF037168); AtJ8, (AF099906); AtJ10, (Y11969) [24]; AtJ11 [23]; AtJ12 (AC00291); AtJ13 (AC005314); AtJ14 (AC005314); AtJ15, (Z49238) [26]; AtJ16, (AF089810) [27]; AtJ17 (AC002396). RLGS, rhodopsin-like GPCR superfamily; ERCG, erythrocruorin family. The RLGS and ERCG signature motifs are described on the Leeds University Bioinformantics Homepage (http://bmbsgi11.leeds.ac.uk/bmb5dp/home.html). A description of coiled-coil domains can be found at: http://www/apbiotech.com/protein/protint/.

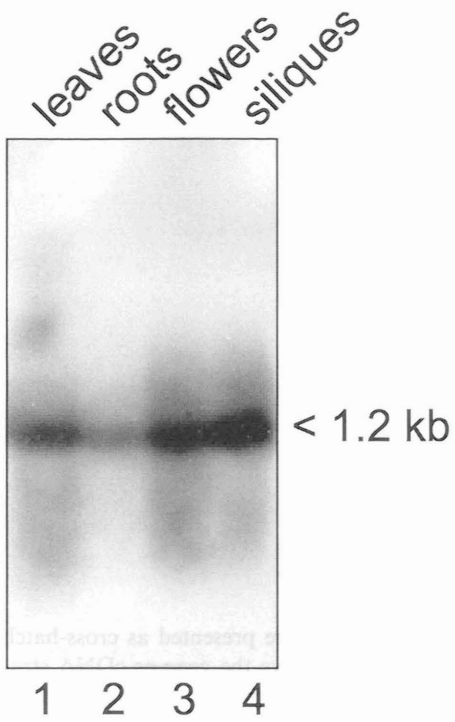

Fig. 4. Expression of the AtJ6 mRNA. Messenger RNAs, isolated from total RNA fractions of leaves (lane 1), roots (2), flowers (3) and siliques (4), were separated on formaldehyde-containing agarose gels, then hybridized with the ^{32}P-labeled cDNA. The carat to the right of lane 4 indicates the position of the 1.2 kb AtJ6 mRNA.

revealing the high degree of sequence conservation that is typical of this class of proteins. This comparison includes sequences from bacteria, yeast, plants and animals, as well as cytoplasmic, mitochondrial, and endoplasmic reticulum-resident proteins.

The G/F domain of DnaJ and homologues is simply a region of approximately 50 amino acid residues immediately downstream of the J-domain that is rich in Gly and Phe residues [14]. *E. coli* DnaJ has 21/50 Gly plus Phe residues, while the *A. thaliana* homologue AtJ2 has 18/50 [12]. It has been proposed that the G/F-domain might serve to regulate target polypeptide specificity, and to modify ATPase activity and chaperone function [16]. The AtJ6 sequence (Fig. 1) has only 5/50 Gly plus Phe residues. The DnaJ zinc-finger motif, thought to directly mediate protein:protein interactions, is characterized by four repeats of the sequence: CxxCxGxG [14]. This motif is absent from the AtJ6 sequence (Fig. 1).

3.3. A region of homology with FtsA

The sequence of AtJ6 less the J-domain was analyzed for clues to cellular function. Several computer algorithms were employed in order to search for sequence motifs involved in protein interactions (i.e., http://apbiotech.com/protein/protint), but none were detected. Homology with other proteins was searched using the various BLAST (Basic Local Alignment Search Tool) algorithms available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The AtJ6 sequence less the J-domain has limited homology with other sequences in the various computer databases. The BLAST searches did, however, identify one region of the AtJ6 sequence that has homology with the prokaryotic cell division protein FtsA (Fig. 2B).

The Fts proteins are components of the cell division apparatus [17–19]. The region of FtsA

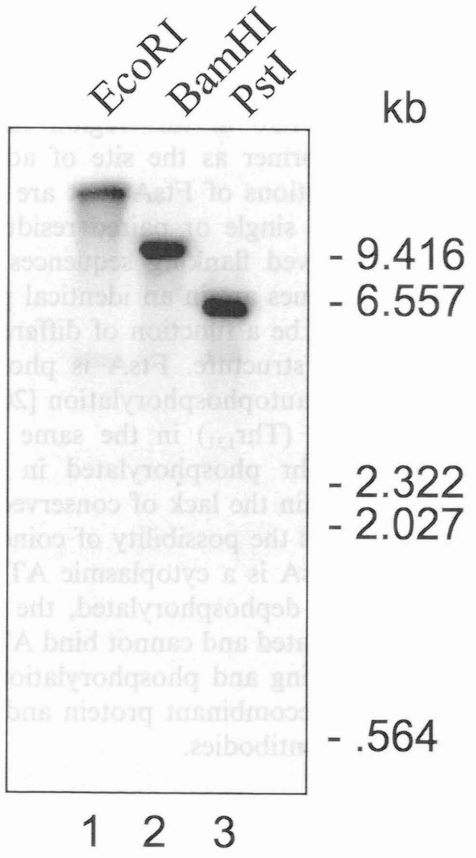

Fig. 5. Southern analysis of AtJ6. Genomic DNA was isolated from light-grown *A. thaliana* seedlings. Ten μg each were digested with *Eco*RI (lane 1), BamHI (lane 2) or *Pst*I (lane 3), transferred to a positively-charged Nylon membrane, and then hybridized with the ^{32}P-labeled cDNA. The positions of size markers are indicated to the right of lane 3.

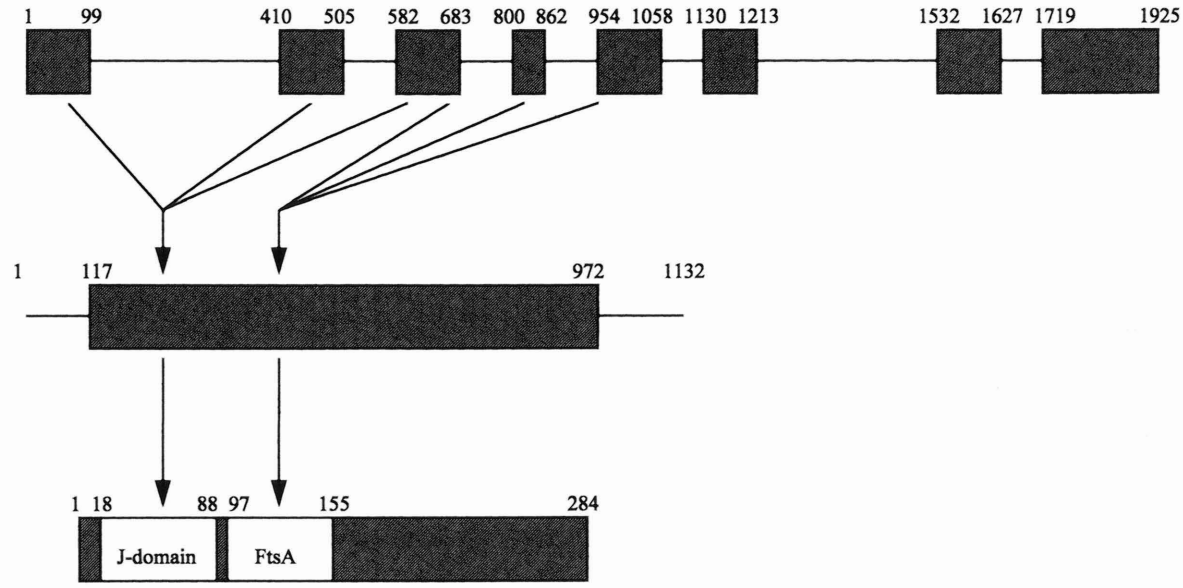

Fig. 6. Schematic representations of the AtJ6 gene, cDNA and protein structures. Exons are presented as cross-hatched boxes, while introns are horizontal lines. The location, in bp, of exons and introns is indicated above the gene or cDNA structure. The location of amino acid residues corresponding to protein sequence domains is indicated above the cross-hatched box. The relative positions of the J-domain and the region of FtsA homology in the gene, cDNA and protein are indicated by the arrows.

homology in AtJ6 is part of the ATP-binding site [18,19]. The greatest sequence conservation between FtsA and AtJ6 in this region has been identified in the former as the site of adenosine binding. Other portions of FtsA that are in contact with ATP are single or paired residues that lack highly conserved flanking sequences. While none of these residues are in an identical position in AtJ6, this could be a function of differences in protein secondary structure. FtsA is phosphorylated, possibly via autophosphorylation [20]. AtJ6 has a Thr residue (Thr_{131}) in the same relative position as the Thr phosphorylated in *E. coli* FtsA, although again the lack of conserved flanking sequences raises the possibility of coincidence. Phosphorylated FtsA is a cytoplasmic ATP-binding protein. When dephosphorylated, the protein is membrane associated and cannot bind ATP [20]. Tests of ATP binding and phosphorylation await expression of the recombinant protein and preparation of suitable antibodies.

3.4. The *A. thaliana* family of J-domain proteins

The only well understood roles for DnaJ and DnaJ homologues are as molecular chaperones or co-chaperones [7]. In this context, the participation of the J-, G/F- and zinc finger-domains have been characterized [14]. Increasingly, whole genome sequencing projects are generating data suggesting J-domain proteins that lack some or all of the other functional domains. A terminology for J-domain proteins has been recently proposed [14]. In this system, type I homologues have all three of the characteristic DnaJ domains, type II homologues have the J-plus G/F- or zinc finger domains, and type III homologues have only the J-domain. AtJ6 is a type III homologue. The only defined function for the J-domain is in directing interactions with the Stress70 and target polypeptide components of the chaperone machine [15].

A schematic display of the *A. thaliana* J-domain family of proteins described to date is presented in Fig. 3. AtJ2 [12] and AtJ3 [13] are type I J-domain proteins that partition between the cytoplasm and the outer surface of organellar membranes [21,22]. AtJ4/5/7/9 are very similar in sequence and organization to AtJ2/3 (J.A.M., unpublished). AtJ1 [11] is a type II J-domain protein and is located within the mitochondrial matrix. AtJ6 has a J-domain but lacks the G/F- and zinc finger domains (Figs. 1 and 2A) and is thus a type III protein [14]. AtJ6 has a region of FtsA homology (Fig. 2B) where type I proteins have the G/F-domain (Fig. 3).

AtJ8/11/14 are very small type III J-domain proteins, encoding little more than the J-domain itself (Fig. 3). In the cases of AtJ8 (in preparation)

and AtJ11 [23], both of which are plastid-localized, the J-domain is preceded by a transit peptide. The AtJ14 sequence contains no obvious subcellular localization information.

AtJ10 [24] is a large type III J-domain protein related to *S. cerevisiae* CAJ1p [25]. While it was suggested that AtJ10 is a potential CaM-binding protein, the sequence does not contain any obvious CaM-binding motifs. CAJ1p binds CaM indirectly via its interaction with the bona fide CaM-binding protein Stress70 [25]. AtJ12, sequenced as part of the *A. thaliana* genome project, is another large type III J-domain protein related to CAJ1p (Fig. 3).

AtJ13/15/16/17 are also large type III J-domain proteins (Fig. 3). AtJ15 was isolated using a yeast genetic screen for proteins that aid tolerance of oxidative stress [26]. The mechanism for this activity is unknown. In addition to the J-domain, the AtJ15 sequence contains rhodopsin-like GPCR superfamily and erythrocruorin family signature sequences (Fig. 3). AtJ13, sequenced as part of the genome initiative, is an AtJ15 isologue. AtJ16 was isolated as Altered Response to Gravity 1 (ARG1) [27]. It has a potential transmembrane sequence followed by a coiled-coil domain. The latter is thought to mediate protein:protein interactions. AtJ17, also sequenced as part of the genome initiative, is an AtJ16 isologue.

The *A. thaliana* J-proteins comprise a large and more diverse family than has been reported from any other organism [8,14], and many of the AtJ proteins are type III homologues. However, even among this group, AtJ6 is unique in having a specific region with high homology to the FtsA cell division-protein (Fig. 2B,Fig. 3). The only known role for protein J-domains is interaction with the Stress70 chaperone machine [7,14,15]. Identification and characterization of proteins that specifically interact with the diverse *A. thaliana* J-domain proteins pose a formidable challenge for plant cell biologists.

3.5. Subcellular localization

The subcellular localization of the AtJ6 protein was examined using the PSORT program (http://www.imcb.osaka-u.ac.jp/nakai/psort.html), version 6.4. This analysis predicts a $>93\%$ probability that AtJ6 is nuclear localized. There are two prominent polybasic motifs in the AtJ6 sequence that could serve as nuclear localization signals [28]; $RKKK_6$ and $RKRKKKK_{215}$ (Fig. 1). It was recently proposed that the J-domain serves to recruit the Stress70 chaperone machine to specific subcellular locations [29]. In this context, one can envision AtJ6 serving as a locus for docking of the remainder of the chaperone machine in the nucleus. The possibility of a nuclear location of AtJ6 makes the potential role for the FtsA-like domain all the more intriguing.

3.6. Expression

DnaJ is a heat shock protein; it is expressed at low level during normal growth but then greatly induced upon heat stress [5]. Many eukaryotic DnaJ homologues are constitutively expressed, and there is less than a 10-fold increase in response to thermal stress [7]. Messenger RNA was separated from the total RNA isolated from various organs of light-grown *A. thaliana*. The mRNA was separated by agarose gel electrophoresis, then hybridized with the ^{32}P-labeled cDNA. A single 1.2 kb band was observed (Fig. 4). The AtJ6 mRNA was expressed in all organs, although the level in roots was relatively low. Northern analysis of plants exposed to a moderate heat shock (1 h at 37°C) showed no significant difference from control plants (data not presented).

3.7. The AtJ6 gene

Single bands were observed when *A. thaliana* genomic DNA was cut with the restriction enzymes *Eco*RI, *Bam*HI or *Pst*I and then hybridized with ^{32}P-labeled AtJ6 (Fig. 5). These results are consistent with AtJ6 being encoded by a single unique structural gene.

While this manuscript was in preparation, a genomic sequence likely corresponding to AtJ6 became available as part of the *A. thaliana* genome project (GenBank Accession AB010697). Based upon comparison with the AtJ6 sequence, the structure of the 1925 bp genomic clone could be deduced (Fig. 6). The AtJ6 gene consists of eight exons interrupted by seven introns. Exons one through three contribute to the protein J-domain, while exons three through five contribute to the region of FtsA homology (Fig. 6). The gene structures of two other *A. thaliana* J-proteins are known, AtJ3 (in preparation) and AtJ12 (Gen-

Bank accession AC002291). While this number of genomic structures is too small for firm conclusions, there does not appear to be any conserved pattern in the number or positions of the introns. It is clear that acquisition of either the J-domain or the region of FtsA homology could not have been accomplished by any simple pattern of sequence acquisition or intron sliding [30].

There are 11 nucleotide differences between our sequence and that determined by sequencing the BAC clone corresponding to a portion of chromosome V. Of these differences, five are silent. It is not clear if the differences are errors by ourselves or the genome researchers, or are due to bona fide polymorphism. The results of our Southern analysis do not, however, indicate a gene family for AtJ6.

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